

QUERY CONTROL FORM		RTIS USE ONLY	
Application No. <u>091724, 876</u>	Prepared by <u>GPB</u>	Tracking Number <u>5985922</u>	
Examiner-GAU <u>Nashed-1652</u>	Date <u>9/23/04</u>	Week Date <u>7/26/04</u>	
	No. of queries <u>1</u>	<u>FW</u>	

JACKET			
a. Serial No.	f. Foreign Priority	k. Print Claim(s)	p. PTO-1449
b. Applicant(s)	g. Disclaimer	l. Print Fig.	q. PTOL-85b
c. Continuing Data	h. Microfiche Appendix	m. Searched Column	r. Abstract
d. PCT	i. Title	n. PTO-270/328	s. Sheets/Figs
e. Domestic Priority	j. Claims Allowed	o. PTO-892	t. Other

[illegible]

E-5 (Rev. 10/01/02)

described in Wu and Kaiser, Dec. 1997, J. Bact. 179(24):7748-7758, was mixed with PCR primers Seq1 and Mxpil1 primers:

Seq1: 5'-AGCGGATAACAATTTACACAGGAAACAGC-3' (SEQ ID NO:3); and

Mxpil1: 5'-TTAATTAAGAGAAGGTTGCAACGGGGGGC-3' (SEQ ID NO:4),

and amplified using standard PCR conditions to yield an ~800 bp fragment. This fragment was cleaved with restriction enzyme KpnI and ligated to the large KpnI-EcoRV restriction fragment of commercially available plasmid pLitmus 28 (New England Biolabs). The resulting circular DNA was designated plasmid pKOS35-71B. --

Please replace the paragraph beginning at page 103, line 4, with the following rewritten paragraph:

-- The sequence of the *pilA* promoter in these plasmids is shown below (SEQ ID NO:5). --

Please replace the paragraph beginning at page 113, line 17, with the following rewritten paragraph:

-- To improve production of epothilones from these vectors, the eryKS5 linker sequences were replaced by epothilone PKS gene coding sequences, and the vectors were introduced into *Streptomyces coelicolor* CH999. To amplify by PCR coding sequences from the *epoA* gene coding sequence, two oligonucleotides primers were used:

N39-73, 5'-GCTTAATTAAGGAGGACACATATGCCCGTCGTGGCGGATCGTCC-3' (SEQ ID NO:6);

and N39-74, 5'-GCGGATCCTCGAATCACCGCCAATATC-3' (SEQ ID NO:7).

The template DNA was derived from cosmid pKOS35-70.8A3. The ~0.8 kb PCR product was digested with restriction enzymes PacI and BamHI and then ligated with the ~2.4 kb BamHI-NotI and the ~6.4 kb PacI-NotI restriction fragments of plasmid pKOS039-120 to construct plasmid pKOS039-136. To make the expression vector for the *epoA*, *epoB*, *epoC*, and *epoD* genes, the ~5 kb PacI-AvrII restriction fragment of plasmid pKOS039-136 was ligated with the ~50 kb PacI-AvrII restriction fragment of plasmid pKOS039-124 to construct the expression plasmid pKOS039-124R. Plasmid pKOS039-124R has been deposited with the ATCC under the terms of the Budapest Treaty and is available under accession number _____. --